

Postprandial responses in hunger and satiety are associated with the rs9939609 single nucleotide polymorphism in FTO

Citation for published version (APA):

den Hoed, M., Westerterp-Plantenga, M. S., Bouwman, F. G., Mariman, E. C., & Westerterp, K. R. (2009). Postprandial responses in hunger and satiety are associated with the rs9939609 single nucleotide polymorphism in FTO. *American Journal of Clinical Nutrition*, 90(5), 1426-1432. <https://doi.org/10.3945/ajcn.2009.28053>

Document status and date:

Published: 01/01/2009

DOI:

[10.3945/ajcn.2009.28053](https://doi.org/10.3945/ajcn.2009.28053)

Document Version:

Publisher's PDF, also known as Version of record

Document license:

Taverne

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

www.umlib.nl/taverne-license

Take down policy

If you believe that this document breaches copyright please contact us at:

repository@maastrichtuniversity.nl

providing details and we will investigate your claim.

Postprandial responses in hunger and satiety are associated with the rs9939609 single nucleotide polymorphism in *FTO*^{1–3}

Marcel den Hoed, Margriet S Westerterp-Plantenga, Freek G Bouwman, Edwin CM Mariman, and Klaas R Westerterp

ABSTRACT

Background: The common rs9939609 single nucleotide polymorphism (SNP) in the fat mass and obesity-associated (*FTO*) gene is associated with adiposity, possibly by affecting satiety responsiveness.

Objective: The objective was to determine whether postprandial responses in hunger and satiety are associated with rs9939609, taking interactions with other relevant candidate genes into account.

Design: Sixty-two women and 41 men [age: 31 ± 14 y; body mass index (in kg/m^2): 25.0 ± 3.1] were genotyped for 5 SNPs in *FTO*, *DNMT1*, *DNMT3B*, *LEP*, and *LEPR*. Individuals received fixed meals provided in energy balance. Hunger and satiety were determined pre- and postprandially by using visual analog scales.

Results: A general association test showed a significant association between postprandial responses in hunger and satiety with rs9939609 ($P = 0.036$ and $P = 0.050$, respectively). Individuals with low postprandial responses in hunger and satiety were overrepresented among *TA/AA* carriers in rs9939609 (*FTO*) compared with *TT* carriers (dominant and additive model: $P = 0.013$ and $P = 0.020$, respectively). Moreover, multifactor dimensionality reduction showed significant epistatic interactions for the postprandial decrease in hunger involving rs9939609 (*FTO*), rs992472 (*DNMT3B*), and rs1137101 (*LEPR*). Individuals with a low postprandial decrease in hunger were overrepresented among *TA/AA* (dominant), *CC/CA* (recessive), and *AG/GG* (dominant) carriers in rs9939609 (*FTO*), rs992472 (*DNMT3B*), and rs1137101 (*LEPR*), respectively ($n = 39$), compared with *TT*, *AA*, and/or *AA* carriers in these SNPs, respectively ($P = 0.00001$). Each SNP had an additional effect.

Conclusions: Our results confirm a role for *FTO* in responsiveness to hunger and satiety cues in adults in an experimental setting. The epistatic interaction suggests that DNA methylation, an epigenetic process, affects appetite. *Am J Clin Nutr* 2009;90:1426–32.

INTRODUCTION

Twin studies have shown a heritability of 40–80% for obesity-related phenotypes (1–3). This implies that genetic variation determines to a large extent whether an individual is prone to develop obesity when food availability is not limited. Because obesity is a complex metabolic disorder, a genetic predisposition for obesity is anticipated to consist of many nucleic variants, all of which exert a small effect. So far, candidate gene studies attempting to identify genetic variants that contribute to the susceptibility for obesity have not been very successful. In this respect, the recent realization of genome-wide association studies has provided researchers with the promising opportunity to identify risk variants for obesity without the need to select

candidate genes a priori. The first series of genome-wide association studies for body mass index (BMI; in kg/m^2) and obesity showed strong and consistent associations with variants in the fat mass and obesity-associated (*FTO*) gene (4–7).

Frayling et al (5) showed that the population attributable risk of obesity and overweight associated with the common rs9939609 in the *FTO* gene is as high as 20% and 13%, respectively. The *A* allele in rs9939609 was shown to not be associated with fetal growth but with a higher BMI and risk of obesity in children from the age of 7 y onward, persisting into adulthood. In young children, the association of the *A* allele with body mass is almost exclusively attributable to changes in fat mass. In adults, individuals with the *AA* genotype weigh 3 kg more on average than do those with the *TT* genotype, and each *A* allele increases BMI by ≈ 0.4 (5). Since then, the association between adiposity and SNPs in *FTO* has been confirmed in many genome-wide association studies (8–12).

The physiologic pathway by which variation in the *FTO* gene influences the risk of developing obesity largely remains to be established. Recent studies have shown that *FTO* is highly expressed in adipose tissue and in hypothalamic areas that are involved in food intake regulation, such as the arcuate, paraventricular, dorsomedial, and ventromedial nuclei (13–15). This suggests that *FTO* may influence adiposity by affecting appetite. Indeed, energy intake from food is higher in children carrying the *TA/AA* genotype in rs9939609 than in *TT* carriers (16, 17). In addition, Wardle et al (18, 19) showed that children aged 8–11 y carrying the *TA/AA* genotype are characterized by a reduced responsiveness to satiety cues, which thereby increases the risk of overeating and developing obesity (20). Whether or not *FTO* affects responsiveness to satiety cues in adults is currently unknown. We hypothesized that hunger and satiety responsiveness is reduced in adult *TA/AA* carriers of the common rs9939609 SNP in *FTO*.

Gerken et al (14) showed that *FTO* may regulate the transcription of genes involved in metabolism by catalyzing DNA demethylation. Moreover, Qi et al (21) suggested that *FTO* likely forms part of a pathway mediating the neuroregulation of

¹ From the Department of Human Biology, Maastricht University, Maastricht, Netherlands.

² Supported by Maastricht University.

³ Address correspondence to M den Hoed, PO Box 616, 6200MD Maastricht, Netherlands. E-mail: m.denhoed@hb.unimaas.nl.

Received May 9, 2009. Accepted for publication August 14, 2009.

First published online September 30, 2009; doi: 10.3945/ajcn.2009.28053.

food intake, with blocking of the leptin signal inhibiting downstream changes in adipose tissue that induce the expression of *FTO*. Given these results, we additionally determined whether postprandial responses in hunger and satiety are characterized by epistatic interactions involving the rs9939609 SNP in *FTO* and common variants in the genes encoding for DNA methyltransferases (*DNMT1* and *3B*) as well as for leptin (*LEP*) and the leptin receptor (*LEPR*). The rationale for the SNPs chosen is provided in Subjects and Methods.

SUBJECTS AND METHODS

Data were collected from intervention studies on the effect of proteins and/or protein contents on the postprandial responses in (an)orexigenic hormones as well as hunger and satiety (22–25). Participants came to the university in the morning and received fixed meals provided in energy balance and according to energy requirements as calculated by using the formula of Harris and Benedict (26). On average, the meals contained 27%, 45%, and 28% of energy from protein, carbohydrate, and fat, respectively. The study conformed to the standards set by the Declaration of Helsinki, and the local Ethics Committee approved the study. Participants provided written informed consent before participating. Recruitment of participants for the last intervention study included in the present report commenced in September 2005. All intervention studies were completed by August 2006.

Postprandial responses in hunger and satiety were determined in 103 individuals of Western European descent (62 women and 41 men aged 31 ± 14 y with a BMI of 25.0 ± 3.1 ; mean \pm SD) by using visual analog scales. After baseline levels were adjusted for, postprandial responses in hunger and satiety were determined. For both hunger and satiety, participants were characterized as having a high or low postprandial response based on 1) the initial rate of the response; 2) the absolute response, that is, the difference between the baseline level and the minimal/maximal postprandial level obtained; and 3) the postprandial area under the curve, measured until 3–4.5 h post-

prandially. For 6 participants, the postprandial area under the curve could not be determined for hunger because of a lack of sufficient data. Therefore, a total of 97 participants were available for this phenotype. A strong postprandial response can either refer to a strong postprandial decrease (for hunger) or increase (for satiety).

DNA isolation and SNP genotyping

Genomic DNA was isolated from peripheral blood leukocytes by using the QIAamp blood kit from Qiagen (Amsterdam, Netherlands). Besides the rs9939609 SNP in *FTO*, SNPs were selected in the *DNMT1*, *DNMT3B*, *LEP*, and *LEPR* genes. To ensure that an ample number of participants were homozygous for the minor alleles, SNPs were only considered candidates when the minor allele frequency in Europeans was $\geq 25\%$, as indicated by the SNP public database (dbSNP; <http://www.ncbi.nlm.nih.gov/SNP>). SNPs that were associated earlier with relevant phenotypes, such as gene expression, protein concentrations, or protein activity, were favored in the selection process. This resulted in the selection of 5 SNPs (Table 1): the aforementioned intronic *A*→*T* SNP in *FTO* (rs9939609), an intronic *A*→*G* SNP in *DNMT1* (rs2290684), an intronic *C*→*A* SNP in *DNMT3B* (rs992472) (27, 28), the -2548G →*A* SNP in the coding region of *LEP* (rs7799039) (29, 30), and the 668A →*G* SNP in *LEPR*, which results in amino acid substitution Gln223Arg (rs1137101) (31–33).

Genotyping was performed by using commercially available TaqMan SNP genotyping assays from Applied Biosystems (Foster City, CA). The procedure was performed according to the manufacturer's protocol and was measured on an Applied Biosystems 7900 HT Fast Real-Time PCR System. Allelic calls were determined semiautomatically by using the allelic discrimination software of Applied Biosystems. On allelic discrimination, 5 samples were randomly selected per genotype for all SNPs to be determined in duplicate. Duplicates were fully concordant with the initial calls.

TABLE 1
Genotypic and allelic distributions according to single nucleotide polymorphism (SNP)

Gene	SNP	Genotype	Frequency	Allele	Frequency	<i>P</i> value ¹
			no. (%)		%	
<i>FTO</i>	rs9939609 <i>T</i> → <i>A</i>	<i>TT</i>	38 (36.9)	<i>T</i>	60.7	0.97
		<i>TA</i>	49 (47.6)	<i>A</i>	39.3	
		<i>AA</i>	16 (15.5)			
<i>DNMT1</i>	rs2290684 <i>G</i> → <i>A</i>	<i>AA</i>	22 (21.4)	<i>A</i>	46.1	0.97
		<i>AG</i>	51 (49.5)	<i>G</i>	53.9	
		<i>GG</i>	30 (29.1)			
<i>DNMT3B</i>	rs992472 <i>A</i> → <i>C</i>	<i>AA</i>	17 (16.5)	<i>A</i>	39.8	0.78
		<i>AC</i>	48 (46.6)	<i>C</i>	60.2	
		<i>CC</i>	38 (36.9)			
<i>LEP</i>	rs7799039 -2548G → <i>A</i>	<i>GG</i>	29 (28.2)	<i>G</i>	51.0	0.38
		<i>GA</i>	47 (45.6)	<i>A</i>	49.0	
		<i>AA</i>	27 (26.2)			
<i>LEPR</i>	rs1137101 668A → <i>G</i> Gln223Arg	<i>AA</i>	29 (28.2)	<i>A</i>	52.9	0.95
		<i>AG</i>	51 (49.5)	<i>G</i>	47.1	
		<i>GG</i>	23 (22.3)			

¹ Obtained by using the chi-square test of Hardy-Weinberg equilibrium. For all SNPs, a 100% success rate was accomplished.

Statistical analysis

Descriptive analysis

Postprandial responses were dichotomized by using sex- and study-specific median values, resulting in an approximately equal number of participants with a high and low postprandial response for each phenotype, meanwhile taking sex and study into account (0 = high postprandial response, 1 = low postprandial response). Age and BMI were not normally distributed among participants. Therefore, the Kruskal-Wallis and the Mann-Whitney *U* tests were used to determine the associations between BMI and rs9939609 and between the postprandial responses and BMI, respectively. These variables were subsequently log transformed for further analyses.

Association analysis

Before the association analysis was performed, the effect of potential covariates (age and BMI) was evaluated for the postprandial responses in hunger and satiety. Covariates were incorporated into the model when *P* < 0.05. A general association test was performed without assuming a mode of inheritance by entering the genotype in the optimized model (with the significant covariates) as 2 class variables. The mode of inheritance was further investigated by testing an additive, dominant, and recessive model. *P* values, odds ratios (ORs), and 95% CIs are provided for the associations shown. All statistical analyses were performed with SPSS 13 for Macintosh OS X (SPSS Inc, Chicago, IL).

To identify epistatic interactions, multifactor dimensionality reduction (MDR) was used. MDR is a frequently used multilocus method that was applied as previously described by Heidema et al (34). Briefly, we applied the MDR software (<http://www.epistasis.org>) to our data set, which comprised an additional 9 control SNPs for this analysis (35), using 10-fold cross-validation to determine the best model for main SNP-SNP effects. The 10-fold cross-validation was repeated 10 times with the use of a different seed value each time to protect against chance divi-

sions of the data set. Finally, applying the MDR permutation module, we tested the significance of the testing accuracy of the best model by forming 1000 data sets with the case status permuted randomly. This way, we validated for each phenotype whether the model was significantly associated with responder status. Subsequently, genotype combinations with similar postprandial response patterns were pooled for the significant models, and logistic regression was used to obtain a statistical interpretation of the results (34).

RESULTS

The genotypic and allelic distributions of the determined SNPs are provided in Table 1. All SNPs were in Hardy Weinberg equilibrium, and BMI was similar between genotypes for all SNPs, including the rs9939609 variant in *FTO*. BMI was also similar between responders and nonresponders for the postprandial responses in hunger and satiety. Correction of the associations for age and/or BMI did not change the results.

Single-SNP associations for rs9939609

Baseline values for hunger and satiety were similar between genotypes. ORs for the associations between postprandial responses and the rs9939609 SNP in *FTO* are shown in Table 2. A general association test showed a significant association between the postprandial decrease in hunger (area under the curve) and the rs9939609 SNP (*P* = 0.036). Although we cannot exclude an additive model (*P* = 0.051), a dominant model fits the data best, with individuals with a low postprandial decrease in hunger being overrepresented among *TA/AA* carriers compared with *TT* carriers (*P* = 0.013) (Figure 1A). A significant association was also observed between the absolute postprandial increase in satiety and rs9939609 (*P* = 0.050). An additive model fits the data best, with individuals with a strong postprandial increase in satiety being overrepresented among *TA/AA* carriers in rs9939609 compared with *TT* carriers (*P* = 0.020)

TABLE 2 Associations between postprandial responses in hunger and satiety and the rs9939609 single nucleotide polymorphism (SNP) in *FTO*¹

Phenotype ² and SNP ³	Model ⁴	Odds ratio ⁵	95% CI	<i>P</i> value
Hunger				
rs9939609 (<i>FTO</i>)	Dominant	3.02	1.26, 7.24	0.013
Satiety				
rs9939609 (<i>FTO</i>)	Additive	2.02	1.12, 3.66	0.020
Hunger				
rs9939609 (<i>FTO</i>) and rs992472 (<i>DNMT3B</i>)	Dominant and recessive	5.00	2.11, 11.90	0.00025
rs9939609 (<i>FTO</i>) and rs1137101 (<i>LEPR</i>)	Dominant and dominant	5.49	2.30, 15.16	0.00013
rs9939609 (<i>FTO</i>) and rs992472 (<i>DNMT3B</i>) and rs1137101 (<i>LEPR</i>)	Dominant, recessive, and dominant	8.06	3.15, 20.41	0.000013
rs9939609 (<i>FTO</i>)	Dominant	3.83	1.49, 9.80	0.0052
rs992472 (<i>DNMT3B</i>)	Recessive	4.03	1.10, 14.9	0.036
rs1137101 (<i>LEPR</i>)	Dominant	3.17	1.11, 9.01	0.030

¹ The results shown are relative to individuals homozygous for the protective alleles (*TT* for *FTO*, *AA* for *DNMT3B*, and *AA* for *LEPR*).
² Phenotype, postprandial response in hunger or satiety; hunger, the postprandial area under the curve for hunger; satiety, the absolute postprandial increase in satiety, that is, the absolute difference between the baseline value and the maximal postprandial value obtained.
³ The SNP included in the logistic regression model.
⁴ The model of inheritance fitting the data best.
⁵ The odds ratio for having a low postprandial response.

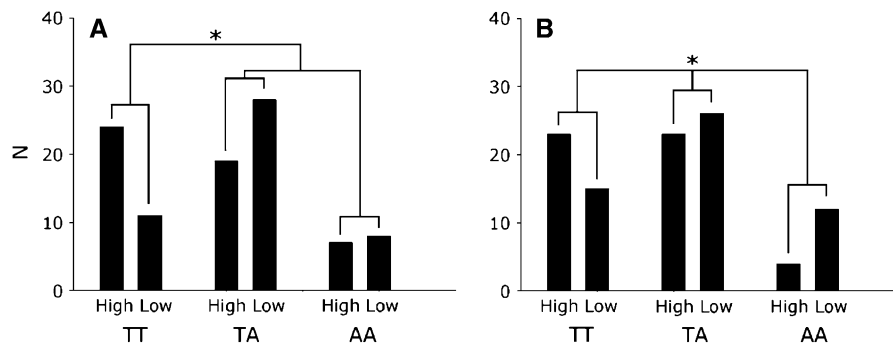


FIGURE 1. Postprandial responses in hunger and satiety as a function of the rs9939609 single nucleotide polymorphism in *FTO*. The numbers of individuals with a high and low postprandial response in hunger (A; dominant model) and satiety (B; additive model) are expressed as a function of genotype in rs9939609. * $P < 0.05$.

(Figure 1B). However, a dominant ($P = 0.065$) or recessive ($P = 0.049$) model cannot be excluded for the latter association.

No significant associations were observed between postprandial responses in hunger and satiety and the SNPs in *DNMT1* and *LEP*. As for the rs992472 (*DNMT3B*) and rs1137101 (*LEPR*) SNPs, individuals with a low postprandial decrease in hunger were overrepresented among CC/CA carriers in rs992472 (*DNMT3B*) compared with AA carriers (recessive model: $P = 0.036$; OR: 3.395; 95% CI: 1.009, 11.416) and among AG/GG carriers in rs1137101 (*LEPR*) compared with AA carriers (dominant model: $P = 0.013$; OR: 3.502; 95% CI: 1.307, 9.382).

Epistatic interactions involving rs9939609

MDR showed 2 significant epistatic interactions for the postprandial decrease in hunger (area under the curve) involving rs9939609. These interactions are in the same direction as the aforementioned univariate associations. One interaction is shown with rs992472 (*DNMT3B*) and the other with rs1137101 (*LEPR*) [prediction accuracy: 66% ($P = 0.050$) and 68% ($P = 0.040$), respectively]. Logistic regression showed that individuals with a low postprandial decrease in hunger were overrepresented among individuals who carried the TA/AA genotype in rs9939609 (dominant model) as well as the CC/CA genotype in rs992472 (*DNMT3B*) (recessive model) compared with carriers of the TT (rs9939609) and/or AA (rs992472) genotype in these SNPs ($P = 0.00025$) (Table 2, **Figure 2**). Individuals with a low postprandial decrease in hunger are also overrepresented among individuals carrying the TA/AA genotype in rs9939609 (dominant model) and the AG/GG genotype in rs1137101 (*LEPR*) (dominant model) compared with carriers of the TT (rs9939609) and/or AA (rs992472) genotype in these SNPs ($P = 0.00013$) (Table 2, **Figure 3**). An evaluation of the combined effect of the 3 SNPs showed that individuals with a low postprandial decrease in hunger were significantly overrepresented among risk-allele carriers in all 3 SNPs, that is, individuals with the TA/AA, CC/CA, and AG/GG genotypes in rs9939609, rs992472, and rs1137101, respectively, compared with individuals homozygous for at least one protective allele in these SNPs (the TT, AA, or AA genotype in rs9939609, rs992472, or rs1137101, respectively) ($P = 0.000013$) (Table 2, **Figure 4**). Each SNP had an additional effect.

DISCUSSION

The main result of the present study was that individuals with low postprandial responses in hunger and satiety are overrepresented among TA/AA carriers in rs9939609 (*FTO*) compared with TT carriers, independent of BMI. This implies that the results of Wardle et al (18, 19), who showed a reduced sensitivity to satiety cues in children with the AA compared with the TT genotype are replicated in adults in an experimental setting. The increased adiposity associated with the TA and AA genotypes in rs9939609 thus appears to at least partly be a function of reduced postprandial responses in hunger and satiety, thereby increasing the risk of overeating (20). The strength of the present study lays in the valid and accurate measurement of the phenotype, that is, the postprandial responses of hunger and satiety. Future studies with larger sample sizes are required to draw firm conclusions about the appropriate mode of inheritance as well as to narrow down the CIs.

In addition to the univariate associations shown for rs9939609, interactions with candidate genes were evaluated to gain more insight into the possible mechanism by which *FTO* affects appetite responsiveness. The association between the postprandial decrease in hunger and the rs9939609 SNP in *FTO* was mediated by the rs992472 variant in *DNMT3B*. Individuals with a low postprandial decrease in hunger are overrepresented among carriers of the TA/AA genotype in rs9939609 (*FTO*) when accompanied by the CC/CA genotype in rs992472 (*DNMT3B*), compared with individuals carrying the TT (rs9939609) and/or AA (rs992472) genotype in these SNPs. *DNMT3B* was originally thought to be responsible for de novo DNA methylation after embryo implantation (27, 36, 37), but was recently concluded to also be essential for the maintenance of preexisting methylation patterns after DNA replication (36, 38, 39).

DNA methylation is a major epigenetic modification that consists of the addition of a methyl group to the 5'-position of cytosine within a CpG dinucleotide, thereby altering gene activity (36). Hypermethylation of the promoter and coding region of a gene decreases its transcription, thereby silencing the gene. Hypomethylation on the other hand enhances the binding of transcription factors, thereby improving gene transcription (27). DNA methylation probably plays a role in body weight regulation, as providing a hypermethylation dietary supplement to Agouti (A^y) mice prevents the transgenerational increase in body weight that occurs without supplementation (40).

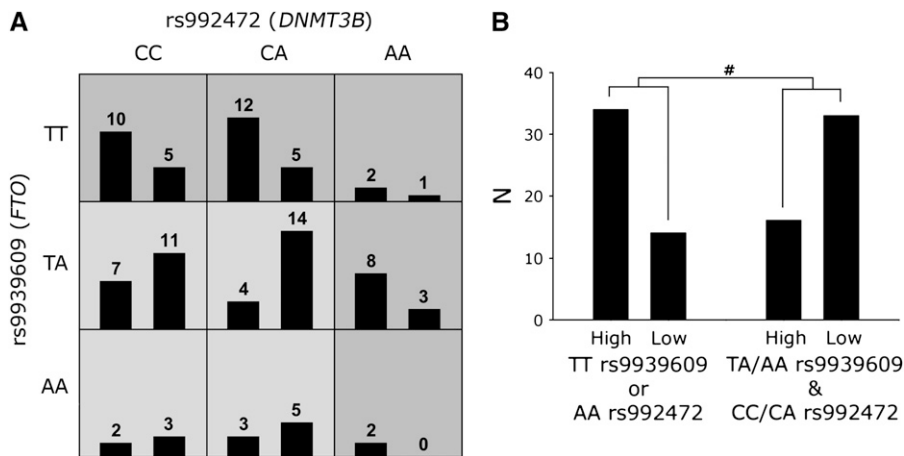


FIGURE 2. Epistatic interaction for the postprandial decrease in hunger involving rs9939609 (*FTO*) and rs992472 (*DNMT3B*). **A:** The output obtained with the use of multifactor dimensionality reduction. The gray squares represent possible combinations of genotypes; the bars on the left represent the number of individuals with a strong postprandial decrease in hunger, and the bars on the right represent the number of individuals with a low postprandial decrease. **B:** The statistical interpretation of the model obtained by using logistic regression. On the basis of the response pattern in panel A, participants carrying the TA/AA genotype in rs9939609 and the CC/CA genotype in rs992472 were pooled and compared with individuals with the TT and/or AA genotype in these single nucleotide polymorphisms, respectively. * $P = 0.00025$.

The rs992472 SNP in *DNMT3B* is located in the same linkage disequilibrium (LD) block as the rs2424913 *C*→*T* SNP in the same gene, for which the minor allele (*T*) is associated with an increased *DNMT3B* promoter activity (28). Additionally, the minor allele in rs2424913 is associated with the risk of developing various types of cancer, probably by aberrant de novo methylation of CpG islands in some tumor suppressor genes (28). Given the LD between rs992472 and rs2424913, a post hoc analysis was performed to determine whether the epistatic interaction for the postprandial decrease in hunger involving rs9939609 (*FTO*) and rs992472 (*DNMT3B*) was confirmed for rs9939609 (*FTO*) and rs2424913 (*DNMT3B*). Because this interaction was indeed observed ($P = 0.002$; data not shown), it is tempting to speculate that a genotype-driven decrease in the capacity for de novo DNA methylation/maintenance of DNA methylation patterns underlies the overrepresentation of in-

dividuals with a low postprandial decrease in hunger among CC/CA carriers in rs992472 (*DNMT3B*) compared with AA carriers.

Fischer et al (41), recently showed that *FTO*^{-/-} mice are characterized by lower adiposity than are their wild-type littermates and concluded that the obesity-predisposing alleles in *FTO* in humans are probably associated with an up-regulation or deregulation of *FTO* expression. In addition, Gerken et al (14) previously showed that *FTO* catalyzes Fe(II) and 2-oxoglutarate-dependent DNA demethylation. These findings suggests that TA/AA carriers in rs9939609 (*FTO*) are characterized by an increased capacity for DNA demethylation compared with TT carriers. The interaction for the postprandial response in hunger involving SNPs in *FTO* and *DNMT3B* could thus result from a decreased capacity for de novo DNA methylation and maintenance of preexisting DNA methylation patterns (CC/CA

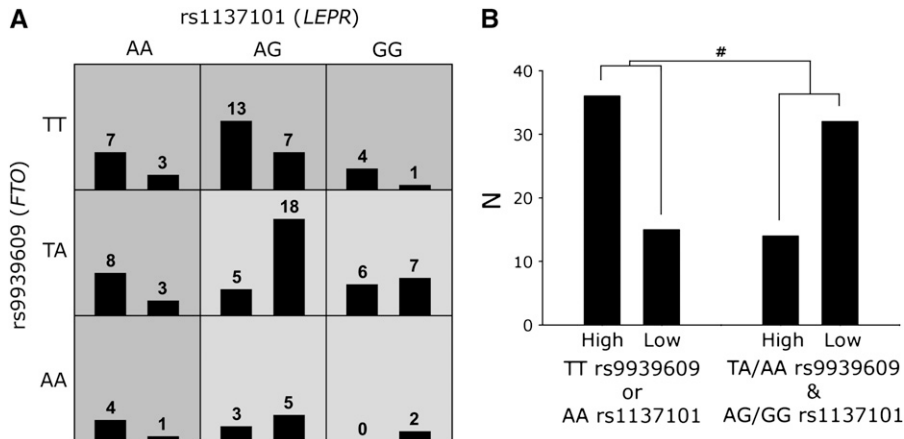


FIGURE 3. Epistatic interaction for the postprandial response in hunger involving rs9939609 (*FTO*) and rs1137101 (*LEPR*). **A:** The output obtained by using multifactor dimensionality reduction. The gray squares represent possible combinations of genotypes; the bars on the left represent the number of individuals with a strong postprandial increase in satiety, and the bars on the right represent the number of individuals with a low postprandial increase. **B:** The statistical interpretation of the model obtained by using logistic regression. On the basis of the response pattern in panel A, participants carrying the TA/AA genotype in rs9939609 and the AG/GG genotype in rs1137101 were pooled and compared with individuals with the TT and/or AA genotype in these single nucleotide polymorphisms, respectively. * $P = 0.00013$.

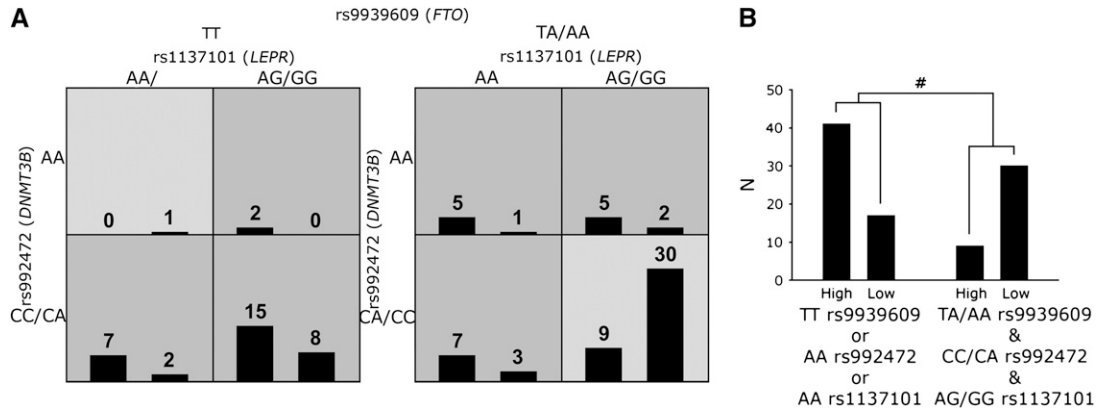


FIGURE 4. Three-way interaction for the postprandial response in hunger. A: The panel on the left shows the epistatic interaction for the postprandial response in hunger involving the rs992472 and rs1137101 single nucleotide polymorphisms (*DNMT3B* and *LEPR*, respectively) for individuals with the *TT* genotype in rs9939609 (*FTO*). The panel on the right shows the interaction for individuals with the *TA/AA* genotype in rs9939609. B: Individuals carrying the *TA/AA* genotype in rs9939609, the *CC/CA* genotype in rs992472, and the *AG/GG* genotype in rs1137101 were pooled and compared with individuals homozygous for at least one protective allele in these single nucleotide polymorphisms, that is, the other genotype combinations combined. $^{\#}P = 0.000013$.

genotypes in rs992472, *DNMT3B*) together with an increased capacity for DNA demethylation (*TA/AA* genotypes in rs9939609, *FTO*), thereby decreasing the overall capacity for DNA methylation. Future studies will have to address whether DNA methylation status is indeed lower in individuals with this genetic background. Furthermore, candidate genes by which DNA methylation affect adiposity will have to be identified (13, 40, 41).

Surprisingly, Fischer et al (41) did not show a difference in food intake between *FTO*^{-/-} mice and their wild-type littermates. Rather, *FTO*^{-/-} mice were characterized by an increased energy expenditure despite a decreased level of physical activity. In humans, most evidence suggests that the association between adiposity and genetic variation in *FTO* results from an effect on energy intake rather than energy expenditure (16–19, 42). If an association with energy expenditure is present at all, the risk allele for obesity appears to be associated with an increased rather than a decreased energy expenditure, resulting from a higher activity-related energy expenditure (16). The apparent discrepancy between humans and mice concerning the mechanism by which genetic variation in *FTO* affects adiposity may result from different candidate genes for DNA methylation being affected differently across species.

The rs1137101 SNP in *LEPR*, resulting in amino acid substitution Gln223Arg, was hypothesized earlier to affect the functionality of the leptin receptor, with the *G* allele (Arg) resulting in reduced leptin sensitivity (31, 33). Resistance to leptin is known to increase food intake, and in extreme cases results in early-onset morbid obesity (43, 44). The interaction for the postprandial decrease in hunger involving SNPs in *FTO* and *LEPR* shows that individuals hypothesized to be leptin resistant are characterized by a blunted postprandial decrease in hunger when also carrying the *TA/AA* genotype in rs9939609. This role for leptin is in line with results from Westerterp-Plantenga et al (45), who showed in obese men consuming an energy-restricted diet that intravenous administration of leptin increases serum leptin concentrations and decreases postabsorptive as well as general feelings of hunger compared with placebo. As for the role of *FTO*, Qi et al (21), hypothesized that blocking leptin may inhibit downstream changes in adipose tissue that induce the

expression of *FTO*. This is in line with the results of Stratigopoulos et al (15), who showed that hypothalamic *FTO* expression is reduced after fasting. However, the fasting-induced decrease in *FTO* expression observed by Stratigopoulos et al was not mediated by leptin. Our results suggest that leptin resistance may only functionally affect *FTO* expression in *TA/AA* carriers.

The interaction between rs9939609 and rs1137101 probably affects the postprandial decrease in hunger via a mechanism other than DNA methylation. After all, if blocking leptin indeed decreases *FTO* expression (21), the risk of obesity is anticipated to be reduced (40, 41), which is the exact opposite of what is expected when the postprandial decrease in hunger is low. This implies that the effect of this alternative mechanism on the postprandial decrease in hunger should not be underestimated.

We did not confirm the association between BMI and the rs9939609 variant shown in previous studies. This can be explained by the range in BMI in our population and by the character of the study and its concomitant sample size. In the current experimental study, 103 participants were recruited with a BMI ranging from 19 to 31. Only 5 individuals with a BMI > 30 were included. This contrasts sharply with case-control studies (6, 46) and with cohort studies (5, 7), in which the association between BMI and rs9939609 was evaluated. In fact, the lack of association between BMI and the rs9939609 SNP is consistent with the results from Frayling et al (5), who did not observe a significant association between BMI and rs9939609 genotype in samples of <1000 individuals. This suggests that BMI, which incorporates both fat mass and fat-free mass, is probably not sensitive enough to be used as a marker for adiposity in genetic studies with a relatively small sample size.

In conclusion, evidence shows an association between postprandial responses in hunger and satiety with the rs9939609 SNP in *FTO*, with the *TA/AA* genotype predisposing individuals to reduced postprandial responses in hunger and satiety. The effect of the variant in *FTO* on the postprandial response in hunger appears to be mediated by an epistatic interaction involving variants in *DNMT3B* and *LEPR*. The interaction with the rs992472 SNP in *DNMT3B* suggests that DNA methylation, an epigenetic process, is involved in food intake regulation.

We thank Kristel Diepvens, Manuela Lejeune, Astrid Smeets, Margriet Veldhorst, and Ananda Hochstenbach-Waelen for their contributions in acquiring the data on postprandial responses in hunger and satiety.

The authors' responsibilities were as follows—MDH: developed the study, performed the genotyping and statistical analyses, and wrote the manuscript; MSW-P, ECMM, and KRW: developed the study and wrote the manuscript; and FGB: performed the genotyping and wrote the manuscript. None of the authors had a conflict of interest.

REFERENCES

- Bell CG, Walley AJ, Froguel P. The genetics of human obesity. *Nat Rev Genet* 2005;6:221–34.
- Faith MS, Pietrobelli A, Nunez C, Heo M, Heymsfield SB, Allison DB. Evidence for independent genetic influences on fat mass and body mass index in a pediatric twin sample. *Pediatrics* 1999;104:61–7.
- Stunkard AJ, Foch TT, Hrubec Z. A twin study of human obesity. *JAMA* 1986;256:51–4.
- Dina C, Meyre D, Gallina S, et al. Variation in FTO contributes to childhood obesity and severe adult obesity. *Nat Genet* 2007;39:724–6.
- Frayling TM, Timpson NJ, Weedon MN, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* 2007;316:889–94.
- Hinney A, Nguyen TT, Scherag A, et al. Genome wide association (GWA) study for early onset extreme obesity supports the role of fat mass and obesity associated gene (FTO) variants. *PLoS One* 2007;2:e1361.
- Scuteri A, Sanna S, Chen WM, et al. Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. *PLoS Genet* 2007;3:e115.
- Loos RJ, Lindgren CM, Li S, et al. Common variants near MC4R are associated with fat mass, weight and risk of obesity. *Nat Genet* 2008;40:768–75.
- Meyre D, Delplanque J, Chevre JC, et al. Genome-wide association study for early-onset and morbid adult obesity identifies three new risk loci in European populations. *Nat Genet* 2009;41:157–9.
- Thorleifsson G, Walters GB, Gudbjartsson DF, et al. Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. *Nat Genet* 2009;41:18–24.
- Waller CJ, Speliotes EK, Loos RJ, et al. Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. *Nat Genet* 2009;41:25–34.
- Lindgren CM, Heid IM, Randall JC, et al. Genome-wide association scan meta-analysis identifies three loci influencing adiposity and fat distribution. *PLoS Genet* 2009;5:e1000508.
- Fredriksson R, Hagglund M, Olszewski PK, et al. The obesity gene, FTO, is of ancient origin, up-regulated during food deprivation and expressed in neurons of feeding-related nuclei of the brain. *Endocrinology* 2008;149:2062–71.
- Gerken T, Girard CA, Tung YC, et al. The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. *Science* 2007;318:1469–72.
- Stratigopoulos G, Padilla SL, Leduc CA, et al. Regulation of Fto/Ftm gene expression in mice and humans. *Am J Physiol Regul Integr Comp Physiol* 2008;294:R1185–96.
- Cecil JE, Tavendale R, Watt P, Hetherington MM, Palmer CN. An obesity-associated FTO gene variant and increased energy intake in children. *N Engl J Med* 2008;359:2558–66.
- Timpson NJ, Emmett PM, Frayling TM, et al. The fat mass- and obesity-associated locus and dietary intake in children. *Am J Clin Nutr* 2008;88:971–8.
- Wardle J, Carnell S, Haworth CM, Farooqi IS, O'Rahilly S, Plomin R. Obesity associated genetic variation in FTO is associated with diminished satiety. *J Clin Endocrinol Metab* 2008;93:3640–3.
- Wardle J, Llewellyn C, Sanderson S, Plomin R. The FTO gene and measured food intake in children. *Int J Obes (Lond)* 2009;33:42–5.
- Schachter S. Obesity and eating. Internal and external cues differentially affect the eating behavior of obese and normal subjects. *Science* 1968;161:751–6.
- Qi L, Kang K, Zhang C, et al. Fat mass-and obesity-associated (FTO) gene variant is associated with obesity: longitudinal analyses in two cohort studies and functional test. *Diabetes* 2008;57:3145–51.
- Diepvens K, Häberer D, Westerterp-Plantenga M. Different proteins and biopeptides differently affect satiety and anorexigenic/orexigenic hormones in healthy humans. *Int J Obes (Lond)* 2008;32:510–8.
- Lejeune MP, Westerterp KR, Adam TC, Luscombe-Marsh ND, Westerterp-Plantenga MS. Ghrelin and glucagon-like peptide 1 concentrations, 24-h satiety, and energy and substrate metabolism during a high-protein diet and measured in a respiration chamber. *Am J Clin Nutr* 2006;83:89–94.
- Smeets AJ, Soenen S, Luscombe-Marsh ND, Ueland O, Westerterp-Plantenga MS. Energy expenditure, satiety, and plasma ghrelin, glucagon-like peptide 1, and peptide tyrosine-tyrosine concentrations following a single high-protein lunch. *J Nutr* 2008;138:698–702.
- Veldhorst M, Smeets A, Soenen S, et al. Protein-induced satiety: effects and mechanisms of different proteins. *Physiol Behav* 2008;94:300–7.
- Harris JA, Benedict FG. A biometric study of basal metabolism in man. Washington, DC: Carnegie Institution of Washington, 1919:279.
- Lee SJ, Jeon HS, Jang JS, et al. DNMT3B polymorphisms and risk of primary lung cancer. *Carcinogenesis* 2005;26:403–9.
- Shen H, Wang L, Spitz MR, Hong WK, Mao L, Wei Q. A novel polymorphism in human cytosine DNA-methyltransferase-3B promoter is associated with an increased risk of lung cancer. *Cancer Res* 2002;62:4992–5.
- Hoffstedt J, Eriksson P, Mottagui-Tabar S, Arner P. A polymorphism in the leptin promoter region (-2548 G/A) influences gene expression and adipose tissue secretion of leptin. *Horm Metab Res* 2002;34:355–9.
- Mammes O, Betoulle D, Aubert R, Herbeth B, Siest G, Fumeron F. Association of the G-2548A polymorphism in the 5' region of the LEP gene with overweight. *Ann Hum Genet* 2000;64:391–4.
- Chagnon YC, Chung WK, Perusse L, Chagnon M, Leibel RL, Bouchard C. Linkages and associations between the leptin receptor (LEPR) gene and human body composition in the Quebec Family Study. *Int J Obes Relat Metab Disord* 1999;23:278–86.
- Mattevi VS, Zembrzuski VM, Hutz MH. Association analysis of genes involved in the leptin-signaling pathway with obesity in Brazil. *Int J Obes Relat Metab Disord* 2002;26:1179–85.
- Yiannakouris N, Yannakoulia M, Melistas L, Chan JL, Klimis-Zacas D, Mantzoros CS. The Q223R polymorphism of the leptin receptor gene is significantly associated with obesity and predicts a small percentage of body weight and body composition variability. *J Clin Endocrinol Metab* 2001;86:4434–9.
- Heidema AG, Feskens EJ, Doevendans PA, et al. Analysis of multiple SNPs in genetic association studies: comparison of three multi-locus methods to prioritize and select SNPs. *Genet Epidemiol* 2007;31:910–21.
- den Hoed M, Smeets AJ, Veldhorst MA, et al. SNP analyses of postprandial responses in (an)orexigenic hormones and feelings of hunger reveal long-term physiological adaptations to facilitate homeostasis. *Int J Obes (Lond)* 2008;32:1790–8.
- Robertson KD, Wolffe AP. DNA methylation in health and disease. *Nat Rev Genet* 2000;1:11–9.
- Wang J, Bhutani M, Pathak AK, et al. Delta DNMT3B variants regulate DNA methylation in a promoter-specific manner. *Cancer Res* 2007;67:10647–52.
- Kangaspekka S, Stride B, Metivier R, et al. Transient cyclical methylation of promoter DNA. *Nature* 2008;452:112–5.
- Li E, Bestor TH, Jaenisch R. Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell* 1992;69:915–26.
- Waterland RA, Travisano M, Tahiliani KG, Rached MT, Mirza S. Methyl donor supplementation prevents transgenerational amplification of obesity. *Int J Obes (Lond)* 2008;32:1373–9.
- Fischer J, Koch L, Emmerling C, et al. Inactivation of the Fto gene protects from obesity. *Nature* 2009;458:894–8.
- Speakman JR, Rance KA, Johnstone AM. Polymorphisms of the FTO gene are associated with variation in energy intake, but not energy expenditure. *Obesity (Silver Spring)* 2008;16:1961–5.
- Clément K, Vaisse C, Lahlou N, et al. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* 1998;392:398–401.
- Lahlou N, Clément K, Carel JC, et al. Soluble leptin receptor in serum of subjects with complete resistance to leptin: relation to fat mass. *Diabetes* 2000;49:1347–52.
- Westerterp-Plantenga MS, Saris WH, Hukshorn CJ, Campfield LA. Effects of weekly administration of pegylated recombinant human OB protein on appetite profile and energy metabolism in obese men. *Am J Clin Nutr* 2001;74:426–34.
- Do R, Bailey SD, Desbiens K, et al. Genetic variants of FTO influence adiposity, insulin sensitivity, leptin levels, and resting metabolic rate in the Quebec Family Study. *Diabetes* 2008;57:1147–50.